

TEXT SEARCHABLE DOCUMENT

Shaughnessy #128921
Algae

DATA EVALUATION RECORD

00159709

1. Chemical: Dicyandiamide
2. Test Material: Dicyandiamide, techn. pure, a white crystalline powder of Batch 7308 (24-10-84). A cover sheet to the study identifies the compound as SKW 8510 NS.
3. Test Type: Aquatic Plant Growth and Reproduction on the green algae Selenastrum capricornutum.
4. Study ID: Oldersma, H.
The effect of the product Dicyandiamide Tech. Pure, on the growth of the green alga Selenastrum capricornutum. Report No. R85/068. Order No. 13576. TNO. P.O. Box 217, 2600 AE Delft, Netherlands. For: SKW Trostberg AG., Trostberg, Germany. 1985-03-26. Study date: 1985
5. Reviewed by: Zigfridas Vaituzis
Microbiologist
EEB/HED
Signature: *Z. Vaituzis*
Date: 12/13/86
6. Approved by: Ray Matheny
Head, Section I
EEB/HED
Signature: *Ray Matheny*
Date: 2/13/87
7. Conclusions:

The 93.5-hr EC₅₀ to the green alga Selenastrum capricornutum was found to be approximately 1025 ppm. The NEL is estimated at 100 ppm.

The study fulfills the Guidelines requirement for an aquatic plant growth study and is acceptable for use in a risk assessment.
8. Recommendations:
N/A.
9. Background:
N/A.
10. Discussion of Individual Tests:
N/A.



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11. Methods and Materials:

a. Test Organisms: Selenastrum capricornutum

Age/Stage of Maturity: Experimental growth phase inocula of at least 2×10^4 cells per mL medium.

Source: American Type Culture Collection, Rockville, Maryland 20852.

b. Dosage Form:

Route of Administration: Dissolved in test algal medium.

c. Referenced Protocol: OECD Guidelines for Testing of Chemicals 201, adopted June 7, 1984, "Alga Growth Inhibition Test," Paris (1984).

Test Levels: 0.1, 0.32, 0.56, 1.0, 1.8, 3.2, and 10 g/L.

Dose Spacing Factors: 0.3 to 0.6 of next higher dilution.

Number Per Level: 1×10^4 cells mg/L in 100 mL batches, in duplicate.

Test Conditions:

Temperature: 20 °C

Dissolved Oxygen: N/A

pH: "approximately 8"

Test Vessels: 200 mL conical flasks

Aeration: Incubator shaker at 120 rpm.

Photoperiod: continuous below a set of fluorescent lamps of 5 K lux intensity.

Diet Preparation: Algal medium prepared according to OECD guideline 201.

Controls: 1×10^4 cells/mL in 100 mL batches, w/o test substance, four groups; and test substance solution without algae for background "noise".

Measured Test Levels: Nominal test levels used for study.

Observation Period: Once a day for 5 days.

- b. Statistical Analysis: The 93.5 hr EC₅₀ was calculated by means of a parametric model developed by Kooijman et al. (Parametric analysis of population growth in bioassays. Water Research 17, 527-538, 1983). The EC₅₀ was calculated with respect to growth rate.

12. Reported Results:

Effects Criteria: Microscopic determination of morphologic form and growth rate inhibition.

EC₅₀: 2040 ppm with respect to growth rate at 93.5 hours.

NEL: 560 ppm estimated nominally by comparison of the growth curves of the treated algal suspensions with those of the blanks.

Dose Response Data: Adequately presented with study.

Observation Period: 93.5 hours maximum.

Toxic Symptoms: No microscopically observable morphological abnormalities were seen in any of the test concentrations, including the toxic concentrations at the end of the study.

pH: increased by 1.5 units in nontoxic concentration flasks.

13. Study Author's Conclusions/Quality Assurance Measures:

The EC₅₀ of dicyandiamide to the freshwater green alga Selenastrum capricornutum with respect to growth rate was found to be 2.04 g/L with a 95 percent confidence interval of 1.95 to 2.13 g/L.

The no-observed-effect concentration (NOEL) was estimated to be 0.56 g/L nominally.

A signed and dated statement of Quality Assurance and GLP compliance is attached to the study.

14. Reviewer's Discussion and Interpretation of the Study:

- a. Test Procedures: The test procedures are adequate to assess the effect of the test chemical on the test organisms. Minor deviations are as follows:
1. The details of the procedure for preparation of the test substance concentrations are not given.
 2. The pH rose more than one pH unit during the test.

These deviations are minor and are not expected to have an effect on the outcome of the study.

Statistical Methods: An examination of the growth curves supplied in the study indicates that the EC₅₀ and the NEL reported are somewhat higher than expected from the test data. Numbers extracted from the concentration effects curves presented in the study were subjected to an EC₅₀ determination using a computerized version of Stephan's binomial probability, moving averages, and probit methods. The results of the moving average give an approximate EC₅₀ of 1025 ppm (95 percent confidence limits = 934 and 1125). The EC₁₀ is approximately 270 ppm, placing the NEL at approximately 100 ppm. Data presented in tables B1 through B4 were also analyzed.

Printouts of the statistical analyses are attached to this DER.

- c. Discussion/Results: The test data subjected to Stephan's moving average test show a 93.5 hr EC₅₀ at 1025 ppm (95 percent confidence limits: 934 and 1125). Examination of the data presented in the study shows the NEL to be approximately 100 ppm.
- d. Adequacy of Test:
 - 1. Validation Category: Core.
 - 2. Rationale: The study fulfills Guidelines requirements.
 - 3. Reparability: N/A.
- 15. Completion of Line-Liner for Test: December 18, 1986.
- 16. CBI Appendix: N/A.

Z.VAITUZIS DICYANDIAMIDE ALGAE 12-18-86

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
10000	475	450	94.73685	0
5000	475	435	91.57895	0
1000	475	200	42.10527	0
500	475	50	10.52632	0
100	475	30	6.315789	0
10	475	0	0	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1251.018

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
5	1.525793E-03		1024.072	933.7144 1125.233

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.2092307	24.91927	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 1.882033
95 PERCENT CONFIDENCE LIMITS = 1.021158 AND 2.742907

LC50 = 1288.815
95 PERCENT CONFIDENCE LIMITS = 674.5843 AND 2425.538

LC10 = 272.5137
95 PERCENT CONFIDENCE LIMITS = 58.70008 AND 547.6556

z.vZITUZIS DICYANDIAMIDE ALGAE TABLE B3 12-18-86

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
10000	480	478	99.58334	0
3200	480	471	98.125	0
1800	480	446	92.91666	0
1000	480	199	41.45833	0
600	480	56	11.66667	0
300	480	31	6.458333	0
100	480	0	0	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1087.545

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
5	2.463551E-03		953.6991 908.9194 1001.784

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	.4486491	60.69468	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 3.93614
95 PERCENT CONFIDENCE LIMITS = 1.299663 AND 6.572616

LC50 = 1001.394
95 PERCENT CONFIDENCE LIMITS = 580.2334 AND 1693.419

LC10 = 476.6183
95 PERCENT CONFIDENCE LIMITS = 88.97808 AND 745.9028

z.vZITUZIS DICYANDIAMIDE ALGAE TABLE B1 12-18-86

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
10000	489	487	99.59101	0
3200	489	480	98.15951	0
1800	489	468	95.70552	0
1000	489	243	49.69326	0
600	489	70	14.31493	0
300	489	40	8.179958	0
100	489	15	3.067485	0

THE BINOMIAL TEST SHOWS THAT 1000 AND 1800 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1003.12

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS		
5	3.117078E-03		838.8798	795.3676	885.2931

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.6948235	101.6456	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 3.221439
95 PERCENT CONFIDENCE LIMITS = .5361741 AND 5.906704

LC50 = 882.1159
95 PERCENT CONFIDENCE LIMITS = 276.719 AND 2271.398

LC10 = 355.8707
95 PERCENT CONFIDENCE LIMITS = 2.440051 AND 671.8767

z.vZITUZIS DICYANDIAMIDE ALGAE TABLE B2 12-18-86

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
10000	466	464	99.57082	0
3200	466	457	98.06866	0
1800	466	426	91.41631	0
1000	466	161	34.54936	0
600	466	36	7.725322	0
300	466	16	3.433477	0
100	466	0	0	0

THE BINOMIAL TEST SHOWS THAT 1000 AND 1800 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1153.988

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
4	2.683665E-03		1089.227 1045.905 1134.779

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
12	1.311072	164.2854	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 4.408362
95 PERCENT CONFIDENCE LIMITS = -.6393023 AND 9.456025

LC50 = 1099.771
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 566.5176
95 PERCENT CONFIDENCE LIMITS = 0 AND 1056.291

z.vZITUZIS DICYANDIAMIDE ALGAE TABLES B1&2 12-18-86

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
10000	478	476	99.58159	0
3200	478	469	98.11716	0
1800	478	445	93.09623	0
1000	478	203	42.46862	0
600	478	54	11.29707	0
300	478	28	5.857741	0
100	478	4	.8368201	0

THE BINOMIAL TEST SHOWS THAT 1000 AND 1800 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1077.617

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS		
5	2.759924E-03		953.1199	905.846	1004.091

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.8166045	111.7576	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 3.760481
95 PERCENT CONFIDENCE LIMITS = .3622782 AND 7.158685

LC50 = 991.8712
95 PERCENT CONFIDENCE LIMITS = 252.0921 AND 3387.77

LC10 = 455.7561
95 PERCENT CONFIDENCE LIMITS = .2163736 AND 818.9731

z.vZITUZIS DICYANDIAMIDE Y 12-18-86 *Algae Table B4*

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
10000	474	473	99.78903	0
3200	474	471	99.36709	0
1800	474	433	91.35021	0
1000	474	204	43.03798	0
600	474	43	9.071729	0
300	474	8	1.687764	0
100	474	0	0	0

THE BINOMIAL TEST SHOWS THAT 1000 AND 1800 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1076.604

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
5	.2800866	1009.299	993.5637 1028.994

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
8	5.432992	643.9008	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 4.931256
95 PERCENT CONFIDENCE LIMITS = -6.5629 AND 16.42541

LC50 = 1045.213
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 577.6486
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY
